Increased mRNA expression and putative release of neuropeptide S after REM sleep deprivation in rat

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Introduction

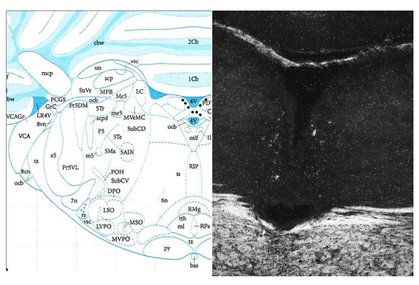
Neuropeptide S (NPS) is a recently described 20 amino acid neurotransmitter /neuromodulator. NPS promotes arousal and its central administartion results anxiolytic effect (Xu YL, 2004). NPS expressing neurons can be found in the brainstem in distinct cell clusters but the NPS receptor (NPSR1) is expressed in several forebrain and brainstem regions (Xu YL, 2007). NPS selectively inhibits the evoked release of serotonin and noradrenaline in frontal cortex - derived synaptosome preparation and central administration of NPS enhanced extracellular dopamine level in the medial prefrontal cortex (Raiter L, 2009; Shi W, 2010). However, the target cells of NPS fibers have not been determined yet in more details.

Aim of the study

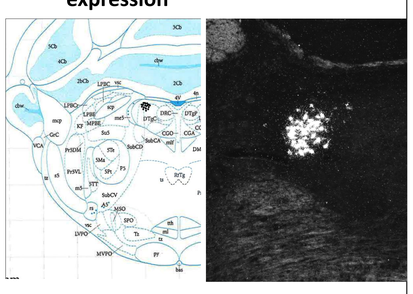
- Detailed anatomical mapping of NPS expressing neurons in the rat brainstem
- Examine the expression of NPS and NPSR1 after REM sleep deprivation and a subsequent rebound sleep.
- A quantitative study on the NPS-immunoreactive (IR) fiber denstity after the sleep deprivation and rebound
- Looking for potential target cells of NPS IR fibers in the hypothalamus with a special attention on catecholaminergic cells.

Results I. Mapping of NPS expressing neurons in the brainstem

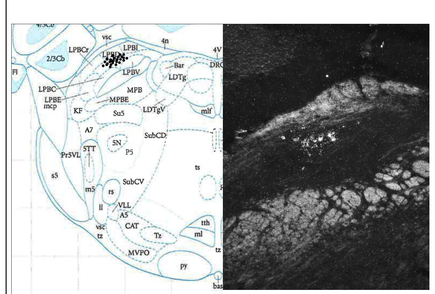
I. Occasional neurons around the fourth ventricle, low expression



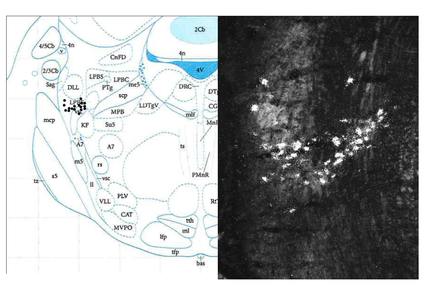
II. Peri-coerulear cluster, high expression



III. Lateral parabrachial nucleus cluster, moderate exprssion

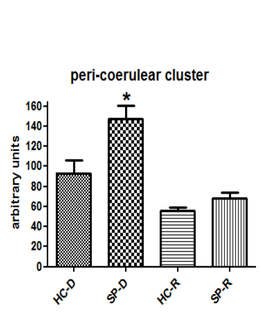


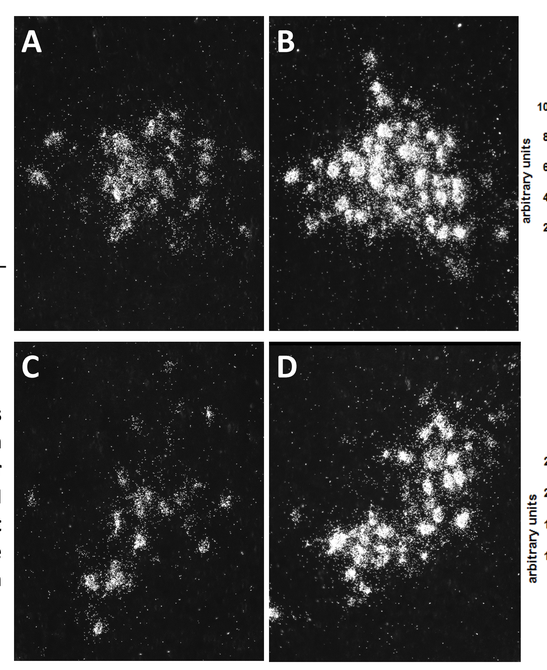
IV. A cell cluster laterally to the external part of lateral parabrachial nucleus and dorso-laterally to the Kölliker-Fuse nucleus (KF cluster), high expression

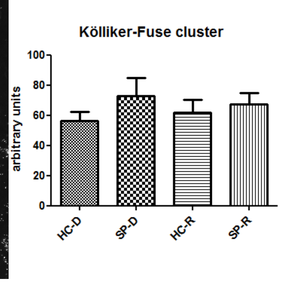


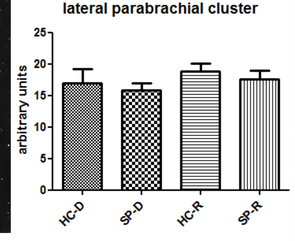
In addition, occasional neurons in the medial parabracial nucleus were also detected (not shown).

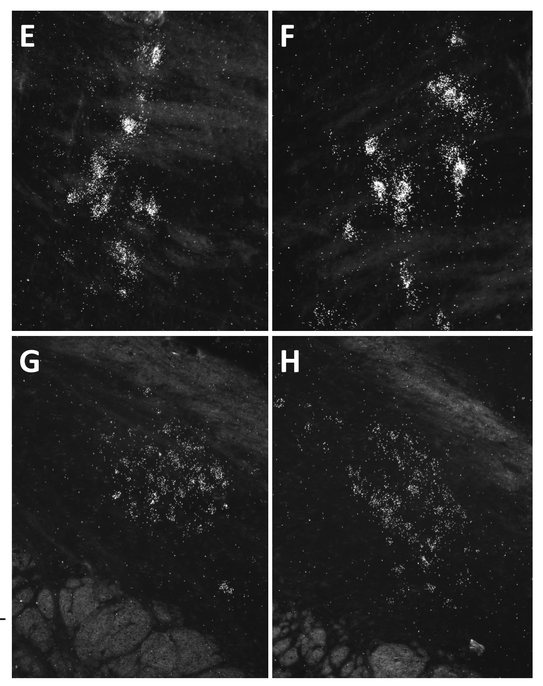
Results II. Cluster-specific alteration of NPS expression during sleep deprivation and rebound sleep









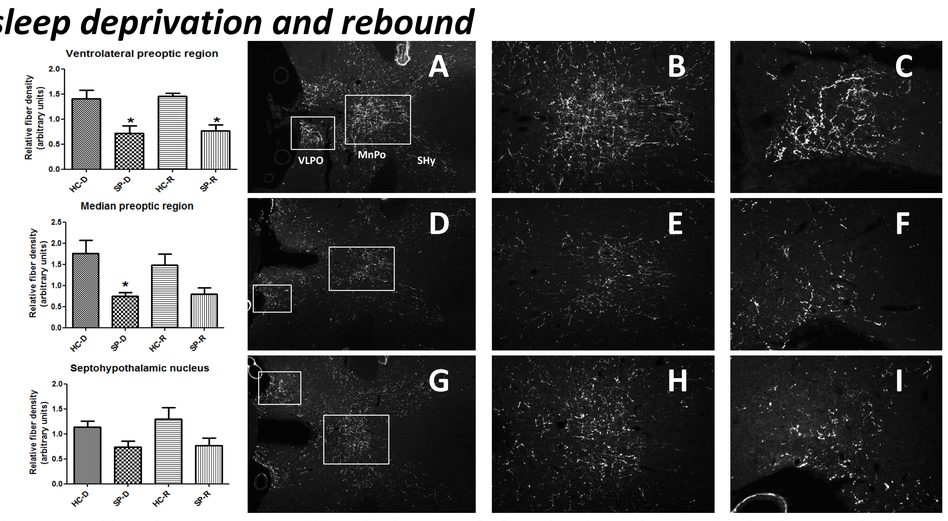


NPS expression is significantly increased in the peri-coerulear cluster after the REM sleep deprivation but returns close to the control level after the 3h rebound sleep.

Peri-coerulear cluster, two rostro-caudal levels (A-D). Homecage for deprived animals (HC-D) (A, C), small pot deprived (SP-D) (B, D), homecage for rebound animals (HC-R), small pot rebound (SP-R). Silver grain countig ; statistical analysis: one-way ANOVA, Tukey post hoc test, N = 5-6, $P < 0.05$. Data are shown as mean ±SEM.

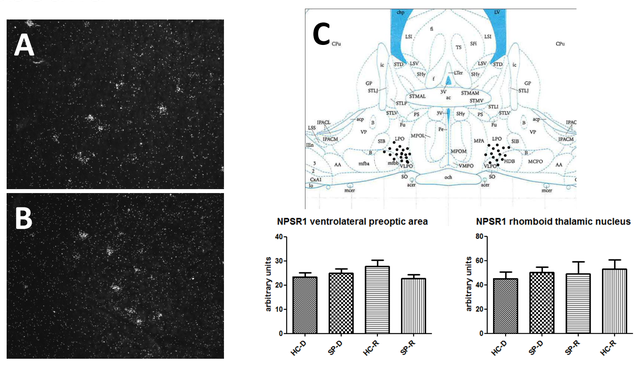
There are no significant alterations in NPS expression in the Kölliker-Fuse cluster (E, F) or in the parabrachial cluster (G, H). Homecage for deprived animals (HC-D) (E, G), small pot deprived (SP-D) (F, H), homecage for rebound animals (HC-R), small pot rebound (SP-R). Silver grain countig ; statistical analysis: one-way ANOVA, Tukey post hoc test, N = 5-6, $P < 0.05$. Data are shown as mean ±SEM.

Results III. Changes in NPS -immunoreactive fiber density after sleep deprivation and rebound



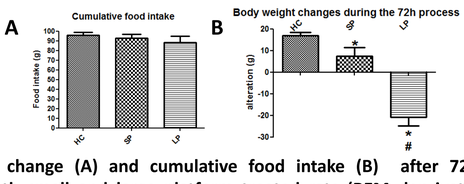
The NPS IR fiber density was significantly decreased after the sleep deprivation in the anterior hypothalamus and the same decrease was noted also after the rebound. Homecage (A-C), small pot deprived (D-F), small pot rebound (G-I). Low power overview of the preoptic region (A, D, G); median preoptic nucleus (MnPo) (B, E, H); ventrolateral preoptic area (VLPO) (G, F, I). SHy: septohypothalamic nucleus. Statistical analysis of fiber densitometry: one-way ANOVA, Tukey post hoc test, N = 4, $P < 0.05$. Data are shown as mean ±SEM.

Results IV. Neuropeptide S receptor 1 expression after sleep deprivation and rebound



The expression of NPS receptor (NPSR1) did not alter significantly in the preoptic region or in the rhomboid thalamic nucleus. Ventrolateral preoptic region (A-C), Homecage for deprived animals (HC-D) (A), small pot deprived (SP-D) (B), homecage for rebound animals (HC-R), small pot rebound (SP-R). Silver grain countig ; statistical analysis: one-way ANOVA, Tukey post hoc test, N = 5-6, $P < 0.05$. Data are shown as mean ±SEM.

Results V. Food intake/body weight alterations during the sleep deprivation process and the potential influence of stress

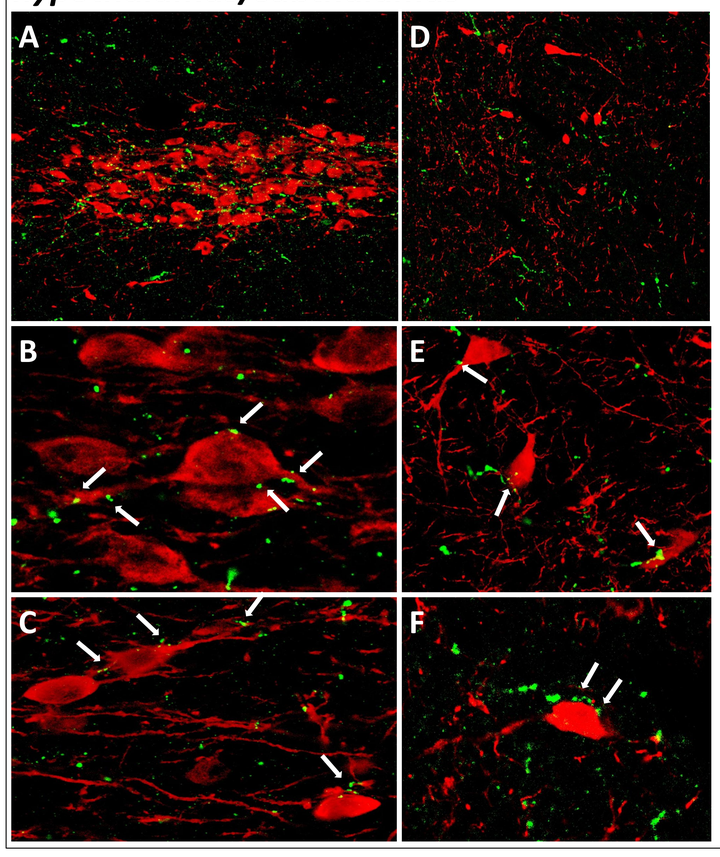


Body weight change (A) and cumulative food intake (B) after 72h spent on platforms. Both small and large platform treated rats (REM deprivation & stress control, respectively) showed a negative energy balance compared to controls (A). There was no difference in the food intake between the groups (B). Data are shown as mean ± SEM, n=5-6, $p<0.05$ *significant difference between HC and pot groups; #significsnt difference between pot groups.

group	*CRF expression (relative optical density measured on film, arbitrary units)*	*NPS expression (grain counting after emulsion, arbitrary units)*
Homecage for deprived	5008±176	73,16±4,95
Small pot deprived (72h)	5683±113*	151,6±15,7*
Large pot deprived (stess control)	5620±85*	77,41±21,19
Homecage for rebound	4966±235	57,5±3,94
Small pot rebound (3h rebound sleep)	5975±90*	65,31±9,89
Large pot rebound (stress control)	5838±219*	73,37±7,14

While the corticotrophin releasing factor (CRF) expression of paraventricular nucleus significantly increased in both platforms, NPS expression elevated only in the the small pot deprived group.

Results VI. Looking for potential targets of NPS fibers in the hypothalamus/subthalamus



Neuropeptide S-positive close contacts on A13 and A15 dopamine neurons in the VLPO or subthalamus, respectively.

A single, 1 μm-thick optical section along the z-plane depicting NPS-containing (*green*) close appositions on TH-positive (*red*) neurons. *Arrows* indicate some of the NPS immunoreactive close contacts on dendrites/somas of DA neurons.

A13 cells, subthalamus, low power micrograph (A); A13 cells with NPS-IR close contacts (B, C); A15 cells in the VLPO, low power micrograph (D); A15 cells with NPS IR close contacts (E, F).

Conclusions

Our results suggest a differential response of NPS expressing neuron clusters after REM sleep deprivation and emphasize the role of the peri-coerulear cluster in the modulation of arousal. This modulation is, however, not associated with changes of NPSR1 expression. The decreased NPS fiber density suggests extensive release of NPS in the preoptic region after sleep deprivation, and there is no restoration during the 3-hour rebound. As the preoptic area is a sleep promoting region, the release of NPS during the forced wakefulness / REM deprivation raises the possibility that NPS facilitates the arousal by an inhibitory action in this region.
The numeral proximal position of NPS IR fibers to the A15/14/13 dopaminergic cell bodies makes these neurons at least one of the potential targets of this action.

Materials & Methods

- Selective REM sleep deprivation was performed on male Wistar rats for 72h by the single platform-on-water method (Kitka T, 2009). The procedure was started and finished at 10.00 a.m., immediatelly after the light change. A subsequent 3h rebound sleep was applied in some groups. Small pot was used for selective REM sleep deprivation. Large pot was used as a stress control. The weight and food intake of animals was monitored. Abbreviations: HC-D: homecage control for deprived animals. SP-D: small pot, deprived animal. HC-R: homecage control for rebound animals. SP-R: REM deprivation + 3h rebound sleep. LP: large pot stress control.
- In situ hybridization with ^{35}S-labeled riboprobes against the rat NPS , NPSR1 and CRF.
- Fluorescent immunohistochemistry for NPS (primer antibody: aNPS rabbit polyclonal (Abcam), secondary antibody: HPR-conjugated labeled polymer (Millipore). Sections were developed with a TSA+ signal amplification kit (Perkin-Elmer).
- Double immunostaining for NPS and tyrosine hydroxylase (TH) / dopamine beta-hydroxylase (DBH). TH /DBH staining was visualized by the incubation of sections with Cy3-conjugated donkey anti mouse/rabbit secondary abs (Jackson immunoresearch). The sections were evaluated by a Zeiss LSM 780 confocal microscope with 488 nm (FITC) and 514 nm (Cy3) excitation lasers.
- Morphometry studies: ISH sections were coated with KODAK NTB2 nuclear track emulsion and the number of silver grains were determined in at least 6 slides from each case (n=5-6) by the ImageJ 1.37v software. The CRF ISH signal was evaluated by measuring the relative optical densitiy of the signal in film. The denstity of NPS IR fibers were determined by the same ImageJ software in at least 6 micrographs / case (N=4) taken by a Nikon Eclipse E600 fluorescent microscope.

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